

**Relationship between fecal score and
oocyst shedding in dairy calves challenged
with *Cryptosporidium parvum***

Honors Thesis

**Presented to the College of Agriculture and Life Sciences,
Department of Animal Science of Cornell University
in Partial Fulfillment of the Requirements for the
Research Honors Program**

by

Meagan Ann Cooney

May 2009

Dr. Daryl Van Nydam, DVM, PhD

Abstract:

Cryptosporidium parvum is a zoonotic protozoan parasite commonly infecting dairy calves less than 30 days old. Infected animals display symptoms of diarrhea and shed approximately 10^7 oocysts / gram of feces. The objective of this study was to determine if there is an association between observed fecal score and oocyst shedding. A double-blinded, randomized trial was conducted using Holstein dairy calves. One of the study personnel was present at all births to transport calves to isolation barns where they were housed for 42 feedings (21d). Calves received 4L of colostrum during their first 12 hours of life and were inoculated with 1.0×10^6 oocysts of *C. parvum* 1-3 hours after the second feeding. Fifty three calves were enrolled in the study (four lost to follow up) and randomly assigned to one of two treatment groups (group 1, n= 25; group 2, n= 24) of a proprietary prophylactic. Each treatment group was analyzed independently. Fecal scores were assigned at each feeding on a scale of 1 to 4, with the higher number indicating increased severity of diarrhea. Fecal samples were collected once daily and analyzed for the presence of *C. parvum* oocysts using the Merifluor Crypto/Giardia immunofluorescence antibody assay. Normal excretion patterns of *C. parvum* were observed and results showed that both treatment groups had a statically significant relationship between the number of oocysts shed in normal feces versus diarrhea. There was no statistical difference in either group for the number of oocysts shed in mild versus severe diarrhea, although the mean number of oocysts shed was increased.

Key words: *Cryptosporidium parvum*, oocysts, fecal score, dairy calves

Acknowledgments:

I would like to begin by thanking Dr. Daryl Nydam for giving me the opportunity to complete this honors thesis project with his research group. He has provided continuous support and guidance to me throughout this study. I would also like to thank my fellow research assistants who helped with calf catching, calf chores, and the preparing and reading of fecal samples. Such individuals include: Dr. Alexandra Burton, Dr. Jenni Zambriski, Isaac Angell, Mary Bellosa, Joshua Boyden, Garth Cummings, Matthew Curler, Karley Hermans, and Tom Linden. A thank you also goes out to the Equine Isolation Barn (EIB) staff consisting of Cliff B., John D., Karyn R., Dan B., and Jackie B. for their help with calf chores. This project was made possible by the funding provided by Schering-Plough Animal Health Corporation. Finally, I would like to thank my family and friends for their love and continuous support in my life's endeavors.

Table of Contents:

<i>Title Page</i>	<i>1</i>
<i>Abstract</i>	<i>2</i>
<i>Acknowledgments</i>	<i>3</i>
<i>Introduction</i>	<i>5-6</i>
<i>Literature Review</i>	<i>6-9</i>
<i>Materials and Methods</i>	<i>10-15</i>
1.1 <i>Animals and Housing</i>	<i>10-11</i>
1.2 <i>Feeding Schedule</i>	<i>11-12</i>
1.3 <i>Excenel Administration</i>	<i>12</i>
1.4 <i>Oocyst Challenge</i>	<i>12</i>
1.5 <i>Sampling Schedule</i>	<i>12-13</i>
1.6 <i>Fecal Sample Analysis</i>	<i>13-14</i>
1.7 <i>Statistical Analysis</i>	<i>14-15</i>
<i>Results and Discussion</i>	<i>15-23</i>
1.1 <i>Average Oocysts Shed vs. Feeding</i>	<i>16-17</i>
1.2 <i>Average Fecal Score vs. Feeding</i>	<i>18-19</i>
1.3 <i>Cryptosporidium parvum oocysts shed vs. fecal score</i>	<i>20-23</i>
<i>Conclusion</i>	<i>24</i>
<i>Literature Cited</i>	<i>24-26</i>

Introduction:

Cryptosporidium parvum is a small (5.0 µm x 4.5 µm) cyst-forming, highly infectious, zoonotic protozoan parasite that is transmitted via the fecal-oral route (Castro-Hermida et. al., 2002; Ollivett et. al., 2009; Saini et. al., 2000). The infectious phase in the lifecycle of this parasite consists of a thick-walled spore, known as an oocyst that is excreted from infected animals (Nydham et. al., 2005). If oocysts are ingested there is a prepatent period of 4-7 days before oocyst shedding begins, after which the duration is approximately 2 weeks (Castro-Hermida et. al., 2002; Olson et. al., 2004). The clinical symptoms of cryptosporidiosis, the disease caused by infection of *C. parvum*, consists of diarrhea that can vary from moderate to intermittent to acute with serious dehydration, depression, anorexia and abdominal pain (Castro-Hermida et. al., 2002; Olson et. al., 2004).

Cryptosporidiosis can be diagnosed by either finding oocysts in the feces of the host species or in histological sections taken during necropsy (Singh et. al., 2006). The pathogen can infect a wide variety of species. However, it mainly affects dairy calves 1-3 weeks of age (Fayer et. al., 1998). Due to this, it has become a major problem in the dairy industry due to economic losses resulting from the high morbidity of calves (de Graff et. al., 1999). In addition, it has also been a worldwide public health concern because of its potential for environmental contamination of surface waters because the oocysts are immediately infective, are extremely resistant to disinfectants, and can remain infective for weeks in the environment (Nydham et. al., 2005). Currently, there are no known effective treatments or vaccines for *C. parvum* (Harp et. al., 1995) except it is likely that

nitazoxanide is effective in reducing the duration of shedding and severity of diarrhea (Ollivett et al., 2009).

Since infected animals are known to respond to *C. parvum* with varying degrees of diarrhea symptoms, we wanted to evaluate if one can identify those animals that are highly infective, since they are a higher risk for contaminating the environment, by simply observing the consistency of their feces. Therefore, the object of this study was to determine if there is an association between fecal score and oocysts shedding.

Literature Review:

Cryptosporidium parvum was first discovered in 1907 by Tyzzer in the gastric glands of the laboratory mouse, but it was not until 1971 it was reported in association with calf diarrhea and 1976 when it was recognized as an enteric human pathogen (Tzipori, 1983; Harp et. al., 1995; Saini et. al., 2000). The infection with *C. parvum* was originally believed to be uncommon, asymptomatic, and highly host specific until the 1980's when it was shown to be an important cause of diarrhea in several species (Tzipori, 1983).

Today, cryptosporidiosis has emerged as a worldwide public health concern being documented in at least 95 countries and infecting a wide variety of hosts including humans and at least 80 species of domestic animals and wildlife (Fayer et. al., 1998; Nydam et. al., 2001; Saini et. al., 2000). However, it is most commonly associated with high morbidity and low mortality in young ruminants, mainly cattle, that are less than 30 days old (Castro-Hermida et. al., 2002).

Due to the hardiness of the oocysts, the infective stage of the parasite, calves are naturally infected when born into a contaminated environment (Ollivett et. al., 2009).

Studies have shown that approximately 60-90% of dairy herds in North America have at least one calf infected with *C. parvum* (Garber et. al., 1994; Trotz-Williams et. al., 2008). When calves are infected with *C. parvum*, dairy farmers have significant economic losses due to the additional costs of treatment, labor and time from the associated symptoms of dehydration and growth retardation (de Graff et. al., 1999).

In humans, *C. parvum* usually causes self-limiting diarrhea in immunocompetent individuals, but can be life-threatening in those with immunodeficiencies (Nydam et. al., 2001; Nydam et. al., 2005). This would include individuals with acquired immune deficiency syndrome (AIDS), those who are malnourished, individuals with concurrent viral infection such as measles, and on occasion, pregnant women (Fayer et. al., 1998).

The lifecycle of *C. parvum* begins with the ingestion of thick-walled oocysts from contaminated food, water or fecal matter. The number of oocysts consumed that will induce infection is approximately 100 (Nydam, 2003). The thick-walled oocysts, which contain four sporozoites, are excysted within the small intestine and invade the epithelial cells of the microvillus brush border. In this location, the sporozoites are in an intracellular and extracytoplasmic position being surrounded by an invagination of the host's cell membrane. This positioning leads to loss of surface area for absorption due to the destruction of the brush boarder, which ultimately leads to malabsorption, maldigestion, and profuse diarrhea (Ollivett et. al., 2009; Saini et. al., 2000; Olson et. al., 2004; CDC, 2008). Within the brush border, the sporozoites undergo asexual reproduction (merogony) and differentiate into the type I meront which produces four haploid merozoites. These merozoites participate in an autoinfection cycle and become known as type II meronts. The type II meronts then go through sexual reproduction

(gametogony), producing microgamonts (male) and macrogamonts (female). Ultimately, these two gametes go through fertilization to produce the oocysts that sporulate within the large intestine. The life cycle of *C. parvum* produces two types of oocysts: ones that are thick-walled and those that are thin-walled. The thick-walled oocysts are the type excreted from the host, whereas the thin-walled oocysts are those that cause autoinfection (CDC, 2008; de Graff et. al., 1999).

The thick-walled oocysts that are excreted into the environment from the host are immediately infective upon shedding (Nydam et. al., 2005). The oocysts are highly resistant and can remain infective for weeks or months in soil or water (Harp et. al., 1995). Since infected animals are known to shed very large numbers of oocysts, approximately 10^7 per gram of feces, this combination allows for easy oocyst transmission to other animals or contamination of water supplies from agricultural run-off (Harp et. al., 1995; Saini et. al., 2000).

C. parvum has been recognized to be one of the most known causes of waterborne gastrointestinal illness in addition to being associated with foodborne outbreaks (MacKenzie et. al., 1994; Quiroz et. al., 2000). Many of the cases have been contributed to surface water of lakes, rivers and streams, well and spring water. Studies have shown that throughout the United States, 65-97% of surface waters are contaminated with *C. parvum* oocysts. In addition, other sources of outbreaks have been linked to swimming pools, amusement park wave pools and water slides (Juranek, 1995). The first waterborne outbreak of cryptosporidiosis occurred in 1984 in Texas from a contaminated artesian well. Another waterborne outbreak occurred in 1993, in Milwaukee, Wisconsin when an estimated 403,000 people showed clinical symptoms of cryptosporidiosis and 54 died due

to waterborne contamination (Saini et. al., 2000). Since the oocysts are rendered inactive by heating for 30 min at 65°C, they do not survive cooking or pasteurization (Saini et. al., 2000; Juranek, 1995). However, food can become contaminated through fecal exposure of infected handlers transmitting oocysts to non-cooked foods such as beverages and salads, or to foods after they have been heated (Juranek, 1995). In addition, freezing and drying methods used in food preservation have been shown to inactivate oocysts to varying degrees (Saini et. al., 2000). Due to its public health concern, since in many of the cases linked to outbreaks the water quality had met state and federal standards, in 1997 *C. parvum* was put on surveillance by the Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA) and USDA Safety and Inspection Service (Juranek, 1995). This surveillance served as a control factor for a possible epidemic outbreak by having laboratory networking to confirm, monitor, and respond to its spread (CDC, 2008).

Since the oocysts are highly resistant, most commercial disinfectants (at recommended levels), including chlorine, are not effective at killing the oocysts. In addition, to date, there are no effective drugs on the market for treatment nor are there any vaccines that have been proven effective in preventing cryptosporidiosis (Harp et. al., 1995; Nydam et. al., 2005). However, a recent study involving the use of nitazoxanide has shown to be effective in reducing the duration of shedding (Ollivett et al., 2009). Treatment of infected individuals or animals is focused on supportive therapy, focusing on rehydration of fluids, electrolytes and energy deficits resulting from the diarrhea (Ollivett et. al., 2009).

Materials & Methods:

1.1 Animals and Housing

In this study, 53 Holstein dairy calves were used in accordance with the Institutional Animal Care and Use Committee (IACUC) and Cornell Center for Animal Resources and Education (CARE) of Cornell University. During the course of the study, from September 2008- February 2009, the calves were housed in the biosecurity level (BSL) II Equine Isolation Barns at Baker Institute of Cornell University, Ithaca NY. The study was double-blinded and randomized into two groups of proprietary cryptosporidiosis prophylactics; group 1 (n=26; bulls n=13, heifers n=13) and group 2 (n=27; bulls n=14, heifers n=13). Since this study was linked with another study, one group was used as the control and the second, unknown, was receiving a proprietary prophylactic treatment. However, for the purpose of this study we observed the overall effects of oocyst shedding, and thus the treatment received is not relevant.

Throughout the time period of the study, three sets of the randomized groups were housed in the isolation barns for a duration of 42 feedings (21d). The calves were obtained from a local dairy farm where at least one of the study personnel attended each calving. At each calving, the perineum of the dam was cleaned using povidone-iodine scrub prior to the catching of the calf at birth onto a single-use plastic sheet or clean wheelbarrow. This technique was performed in order to prevent the calves from contamination with on-farm pathogens. The calves were then moved into a van modified with three washable cages for transportation from the farm to Cornell University's Equine Isolation Barn. Once at the barn a physical exam was performed, an identification tag was placed in the right ear and the umbilicus was dipped with iodine. In addition,

each calf was given 3mL of vitamin E and selenium (BoSe) by an intramuscular injection to prevent white muscle disease, since forage-based diets in the Northeast are selenium deficient. The calves were then individually placed into an 8' x 12' pen inside a well-ventilated room that averaged between 20°-23° Celsius. The pens were cleaned daily by removing wet shavings and fecal matter and filled with wood shavings as needed.

In order to avoid contamination between calves, all study personnel wore disposable coveralls and pull-over boots specific to only one animal. In addition, each calf had their own labeled esophageal tube feeder, milk bottle, milk bucket, water bucket, and thermometer. At the conclusion of the study, the heifer calves were raised as replacements and the bull calves were sold.

1.2 Feeding Schedule

Feeding 1, consisting of 2L of heated colostrum, was administered within the first 1-3 hours of life via an esophageal tube feeder. Feeding 2, also consisting of 2L of heated colostrum, was administered during the calves' first 10-12 hours of life via an esophageal tube feeder. Beginning at feeding 3, calves were fed 4L (0.68 kilograms dry matter) of 20:20 milk replacer daily (2L per feeding) for the remainder of the study. Calves weighing between 36 and 45 kilograms, at an ambient temperature of 20° Celsius, need 0.54 to 0.64 kilograms of dry matter milk replacer per day to meet maintenance requirements and gain 0.45 kg/day based on NRC recommendations, 2001). Study personnel assured that all calves drank the entire amount of milk replacer allotted at each feeding. If not consumed in approximately fifteen minutes after being received, the calves were tube fed the remaining. This was done in order to ensure that nutritional effects were not a variable. Past studies have concluded that calves receiving a higher level of

nutrition can overcome *C. parvum* sooner due to increased growth and feed efficiency (Nydam, Personal Communication, unpublished data 2009). Water was available *Ad libitum*.

1.3 Ceftiofur Administration

In order to prevent *Salmonella*, calves were given ceftiofur (Excenel) for the first five days after birth. Each calf was given a 5 mL of ceftiofur subcutaneously using an 18G needle. It was injected under the skin on the neck and pulling back on the syringe plunger was used to avoid intra-venous/ arterial administration. The first dose was given on farm after birth. The second through fifth doses were given every morning at the barn. Injections were given in alternating sides of the neck for each consecutive dosing.

1.4 Oocyst Challenge

All calves were inoculated with 1.0×10^6 oocysts from a field strain of *C. parvum*, 1-3 hours after feeding 2. The oocysts used in the inoculation were determined by a dye permeability assay to have > 90% viability (Ollivett et. al., 2009). The oocysts were suspended in a 5 mL solution that was given to the calves orally through an esophageal tube feeder. In order to ensure that the entire suspension was given to the calf, the tube was flushed with 120 mL of water.

1.5 Sampling Schedule

At feedings 1 and 4, 10 mL blood samples were taken via jugular vein puncture into plain evacuated tubes. The blood was centrifuged and the serum was collected and measured using a refractometer to obtain serum total proteins (TP).

At each feeding health scores and fecal scores were recorded. Health scores were rated on a 4 point scale; 1 = normal (alert and responsive to feeder), 2= mildly depressed (slow to respond to feeder but eventually does), 3= severely depressed (no response to feeder after multiple attempts), 4= dead or moribund. Fecal scores (FS) were rated on a 5 point scale; 1 = normal (retains form / does not flow across a surface), 2= mild diarrhea (flows slowly across a surface), 3= moderate diarrhea (fairly watery / flows easily across a surface leaving adherent material), 4=severe diarrhea (very watery / leaves no residue when flowing across a surface), 5= not observed. (All of the research assistants on the study were blinded to the group differences when making observations.)

Fecal samples, of at least 10 grams, were collected at feedings 2 and 3, then once daily until the completion of the study. Samples were usually collected at the morning feeding. If however, this was not possible, a sample was obtained at the evening feeding instead. These samples were used to determine oocyst quantification and dry weight measurements.

1.6 Fecal Sample Analysis

Fecal samples collected were analyzed for the presence of *C. parvum* oocyst using the Merifluor Crypto/Giardia immunofluorescence antibody detection reagent from Meridian Diagnostics. The following procedure was obtained from Ollivett et. al., 2009. In a 15 mL conical centrifuge tube, 0.10g of fecal sample was added with 10mL of PBS (pH=7.4). After mixing, 100 μ L was removed and 5 μ L of Merifluor immunofluorescence antibody reagent was added and the solution was vortexed. It was then incubated for at least 30 minutes in the dark at room temperature. After incubation, the solution was stored at 4°C until examination. At the time of viewing, a 10.5 μ L sample

was placed on a slide and covered with a coverslip. The slides were read to count the number of oocysts using the 10x objective lens on a fluorescent compound binocular microscope (460-490 wavelength fluorescent compound binocular microscope Olympus BX41, Olympus America Inc., Center Valley, PA). After reading the slide, the number of oocysts counted was multiplied by 10,000 to obtain the number of oocysts per gram of feces. This value was then standardized using the dry weight percentage. The dry weights of each sample were obtained by drying 10-20g of a fecal sample at 108°C for at least 24 hours and then weighing.

1.7 Statistical Analysis

Averages of fecal scores and oocysts shed for each animal per day were calculated and graphed against its feeding number. The two daily fecal scores obtained per calf per day were averaged together to produce a single score corresponding to the number of oocysts shed from the one fecal sample collected daily. This averaging produced 7 categories of data (1, 1.5, 2, 2.5, 3, 3.5, 4) which were then consolidated into three groups; 1 = normal (1, 1.5), 2= mild diarrhea (2, 2.5, 3), and 3= severe diarrhea (3.5, 4). Box plots, one sample t-tests and ANOVA statistics were run to test the difference in mean number of oocysts shed in each fecal score category. Oocyst shedding data was not incorporated until after the prepatent period; calves that had not begun shedding oocysts, their “0” oocyst count was not included with its associated fecal score so that the overall oocyst averages were not inaccurately lowered. At no point in the data analysis were the two treatment groups combined. Since one of the two groups, unknown because of blinding, received a propriety prophylactic, the differences in the biological activity among the two groups of animals would be too variable to be combined to test the

hypothesis of this study. All data was analyzed using Minitab® 15.1.20.0.statistical software.

Results and Discussion:

The results of this study were based on the analysis of 49 Holstein calves randomly assigned to two groups; group 1 (n=25; bulls n=13, heifers n=12) and group 2 (n=24; bulls n=13, heifers n=11). During the course of the study, four animals were lost due to the death; three from abomasitis and 1 euthanized because of a broken leg. Of the calves that had completed the study, group 1 had an average daily gain (ADG) of 0.24 kg/day and group 2 had an ADG of 0.25kg/day; indicating that the two groups did not differ significantly in weight gain ($p = 0.79$).

In order to ensure that no calves had suckled from their dam or had received colostrum on the farm, passive transfer of maternal antibodies was assessed by measurement of serum total protein (TP) at feedings 1 and 4. At feeding 1, TP was 4.3 g/dL in group 1 and 4.1 g/dL in group 2, indicating that no colostrum was received ($TP < 4.85$ g/dL). In addition, total proteins were measured at feeding 4 to verify that passive transfer was successful ($TP \geq 5.0$ g/dL). Results showed that both groups had acceptable TP levels that did not significantly differ from one another (TP = group 1: 5.1 g/dL; group 2: 5.0 g/dL; $p = 0.621$) indicating that the two groups of calves had similar antibody loads.

Due to one of the groups, unknown because of blinding, receiving a propriety prophylactic, when interpreting the data, one should not compare results between the two groups, but rather the number of oocysts shed within each group.

1.1 Average Oocysts Shed vs. Feeding

Figure 1 illustrates the average number of oocysts shed relative to the feeding number for groups 1 and 2 respectively. In our study, all of the calves were dosed with 1.0×10^6 oocysts at feeding 2, (1d). According to our results, both groups of calves began shedding oocysts on feeding 11/12. In addition, both groups had their peak shedding of oocysts occurring on feeding 23/24. The two groups differed in time point at which oocyst shedding was completed. In group 1, shedding ended at feeding 41, revealing a total duration of shedding lasting 14.5 days. In group 2, oocyst shedding ended at feeding 37/38, giving this group a shedding duration of 13.5 days.

Previous studies have concluded that after calves are exposed to *C. parvum* there is an average incubation period of 5-7 days before oocyst shedding begins (Castro-Hermida et. al., 2002). It has also been observed from past studies that the duration of oocyst shedding lasts 8-23 days (Castro-Hermida et. al., 2002) in which peak shedding occurs around day 14 (Olson et. al., 2005). This data would correspond to approximately our feedings 10-14 to begin shedding, and peak shedding occurring around feeding 28. Besides the calves having peak oocyst shedding occurring at approximately 4-5 feedings ahead of what is seen on average, the data from both calf groups depicted the typical shedding patterns of *C. parvum*.

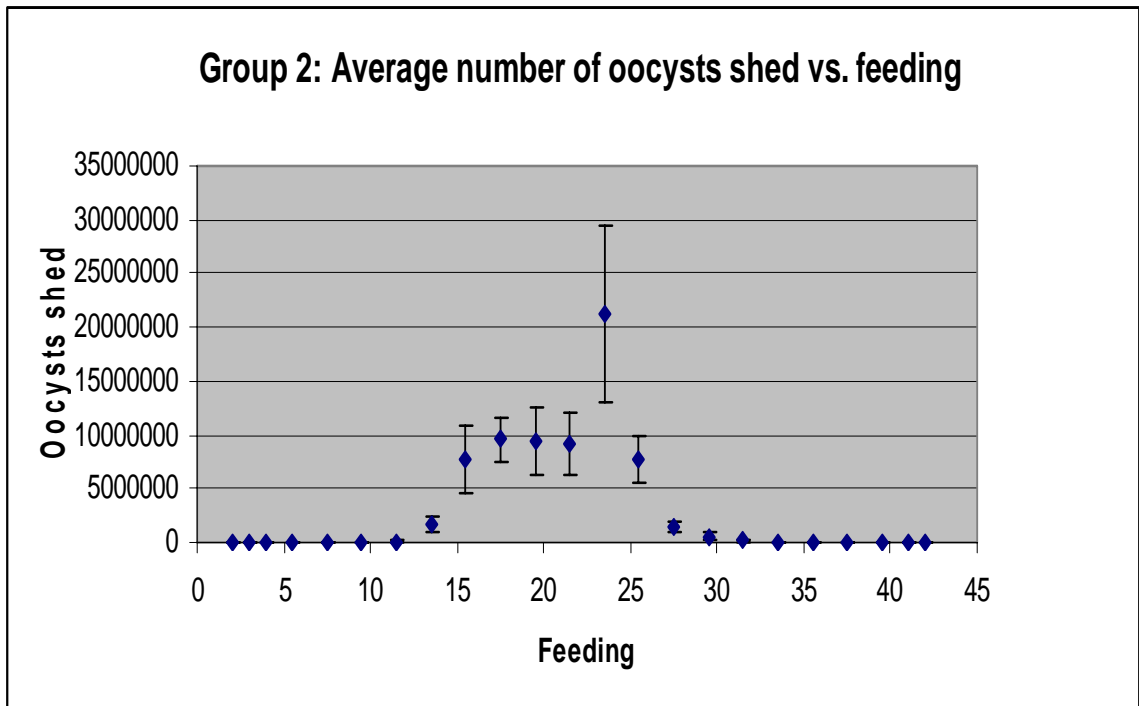
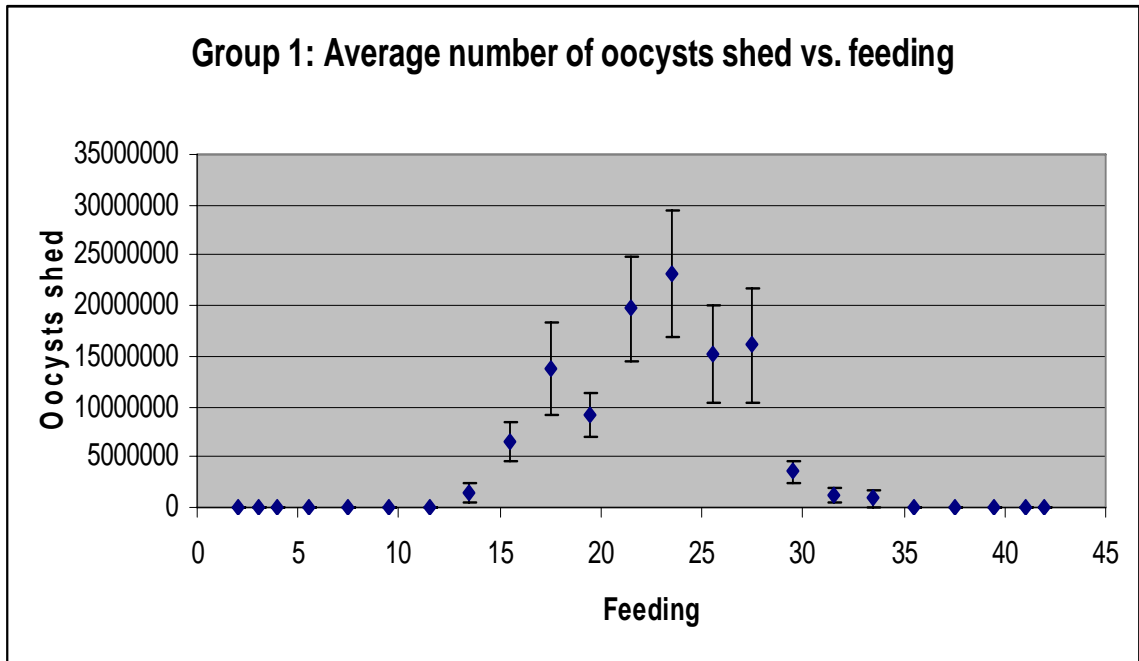


FIGURE 1. The average number of oocysts shed, including standard errors, during each feeding for groups 1 and 2. Fecal samples were collected at every other feeding (1X daily) and analyzed using the Merifluor Crypto/ Giardia immunofluorescence antibody assay to determine the number of oocysts present. Oocyst numbers from all calves for each feeding were averaged together and displayed above.

1.2 Average Fecal Score vs. Feeding

Fecal scores allotted for each feeding per calf were averaged together and the results are displayed in Figure 2 for groups 1 and 2 respectively. Both groups display similar fecal score curves throughout the duration of the study.

Group 1 maintained normal fecal consistency, classified in this study as a fecal score of approximately 1.5, until around feeding 10 when average fecal scores began increasing linearly, peaking at feeding 19. After feeding 19, fecal scores began decreasing toward normal values and reached a value of approximately 1.6 around feeding 33. This value remained stable until the conclusion of the study.

Group 2, exhibited a slight ($FS < 2$), abnormal rise in fecal scores around feeding 4, which eventually decreased back to normal around feeding 10. At this point, fecal scores began to rise linearly until feeding 19. Fecal scores then exhibited an intermediate decrease in fecal scores around feeding 22, which rose again at feeding 25, then began descending to normal, approximately 1.7, around feeding 30.

The duration of diarrhea in the two groups, which was classified as a fecal score >2.0 , span feedings 16-29. This corresponded to 14 feedings or 7 days. Previous studies have concluded that the average diarrhea displayed in calves infected with *C. parvum* lasts between 5-12 days (Castro-Hermida et. al., 2002). Our results of an average of 7 days for both groups indicate that the calves in this study depicted a normal duration of diarrhea corresponding to a *C. parvum* infection.

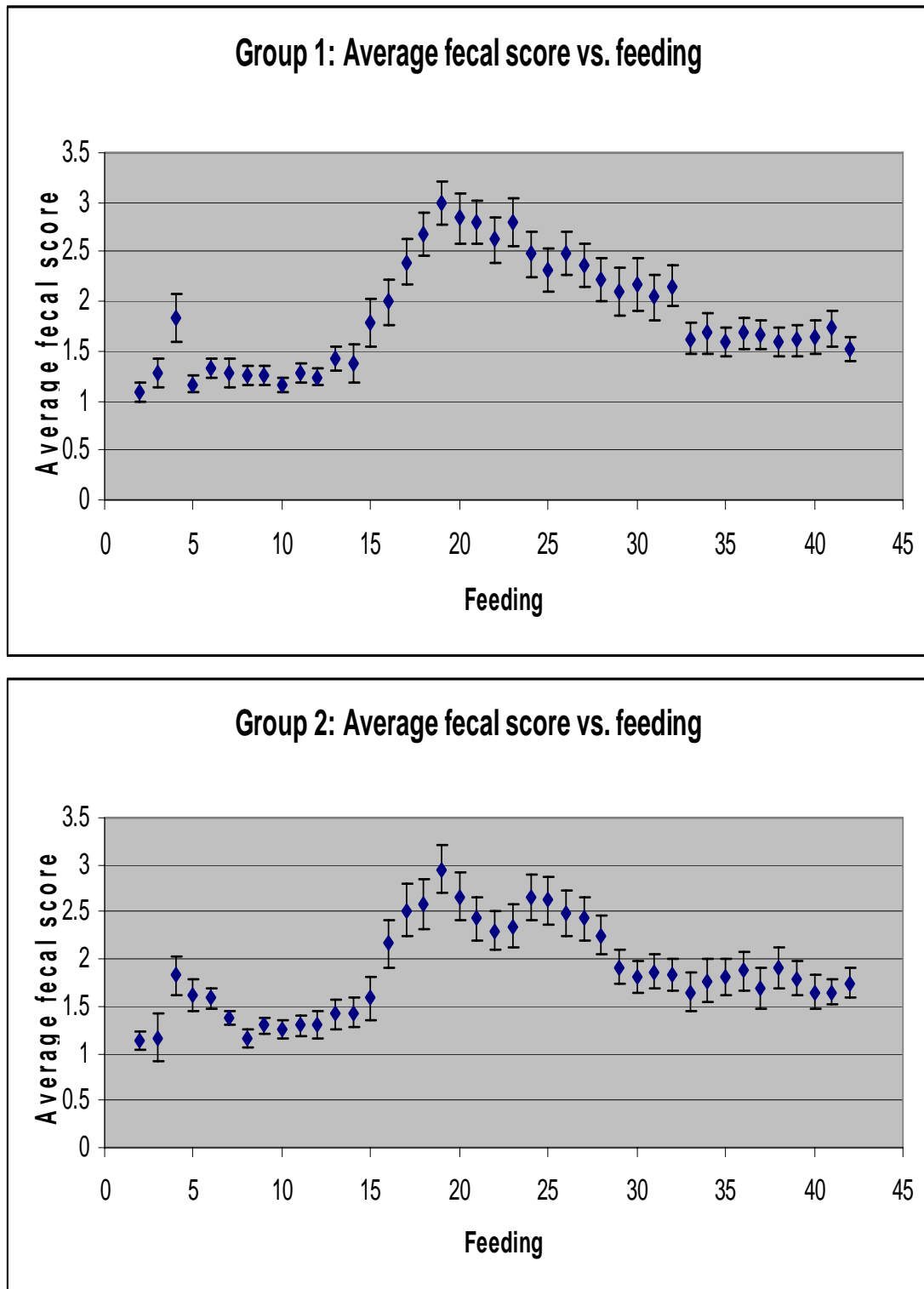


FIGURE 2. The average fecal score, including standard errors, recorded during each feeding for groups 1 and 2. Fecal scores were assigned to fecal matter observed at each feeding on a 1-4 scale; (1 = normal, 2= mild diarrhea, 3= moderate diarrhea, 4=severe diarrhea) Fecal scores from all calves at each feeding were averaged together and displayed above.

1.3 Cryptosporidium parvum oocysts shed vs. fecal score

The number of oocysts obtained from fecal samples, disregarding feeding number, was matched with its corresponding fecal score and descriptive results of each fecal category are displayed in Table 1 and Figure 3 for both groups. Using this data, the relationship between fecal score and the number of oocysts was determined (Table 2).

For group 1, the values comparing the number of oocysts and fecal score were statistically significant for fecal scores 1 versus 2 ($p = 0.005$) and 1 versus 3 ($p = 0.000$). However, results were not statically significant for fecal scores 2 versus 3 ($p = 0.399$) (Table 2).

For group 2, only fecal scores 1 versus 2 were statistically significant ($p = 0.022$). Comparisons for fecal scores 1 versus 3 ($p = 0.079$) and 2 versus 3 ($p = 0.644$) were shown to not be statistically significant even though there was an increase in the mean number of oocyst observed with higher fecal scores (Table 1).

One possible explanation for these results could be due to the smaller sample size in the fecal score 3 category. If this category had a few extremely low or high oocyst numbers it could have had an impact on the overall mean. In fact, in group 2, there was an extremely high outlier present which had an impact on the mean.

Another possible explanation for these results might be observed by looking at peak oocyst shedding (figure 1) and peak fecal scores (figure 2). When observing these two variables, one may notice that they do not overlap. Peak oocyst shedding was seen at feedings 23/24 for both groups whereas peak fecal scores were seen at feeding 19 for both groups. The earlier onset of peak diarrhea relative to peak oocyst shedding might be due to the body's physiological response to the oocyst infection. Once the calf starts to

fight off the infection and fecal score decreases, the number of oocysts reproducing may still be increasing due to autoinfection; thus yielding more oocysts in the feces. This might indicate that peak oocyst numbers may actually be reached during mild rather than severe diarrhea and might explain why fecal scores 2 and 3 did not differ significantly ($p > 0.05$) from one another in either group. Consistent with this, within the first ten feedings there was a rise in fecal score for both groups at feedings 3 and 4 (figure 2) while oocyst shedding did not start until feedings 11 and 12 (figure 1).

These findings suggest that the rise in fecal score observed was not due to factors associated with *C. parvum* shedding. In addition, previous studies have concluded that oocyst shedding does not always begin with the onset of diarrhea (Castro-Hermida et. al., 2002). This would explain why calves that had normal fecal scores also had mean oocyst shedding numbers of 5.53×10^6 and 4.90×10^6 for groups 1 and 2 respectively.

Additional studies observing individual animals' responses to this general shedding trend might be useful. If certain animals do not exhibit a rise in fecal score when shedding *C. parvum*, those animal's should be observed either behaviorally or physiologically to determine why that might be the case. Such differences may reveal why some calves are more asymptomatic than others when infected with *C. parvum*.

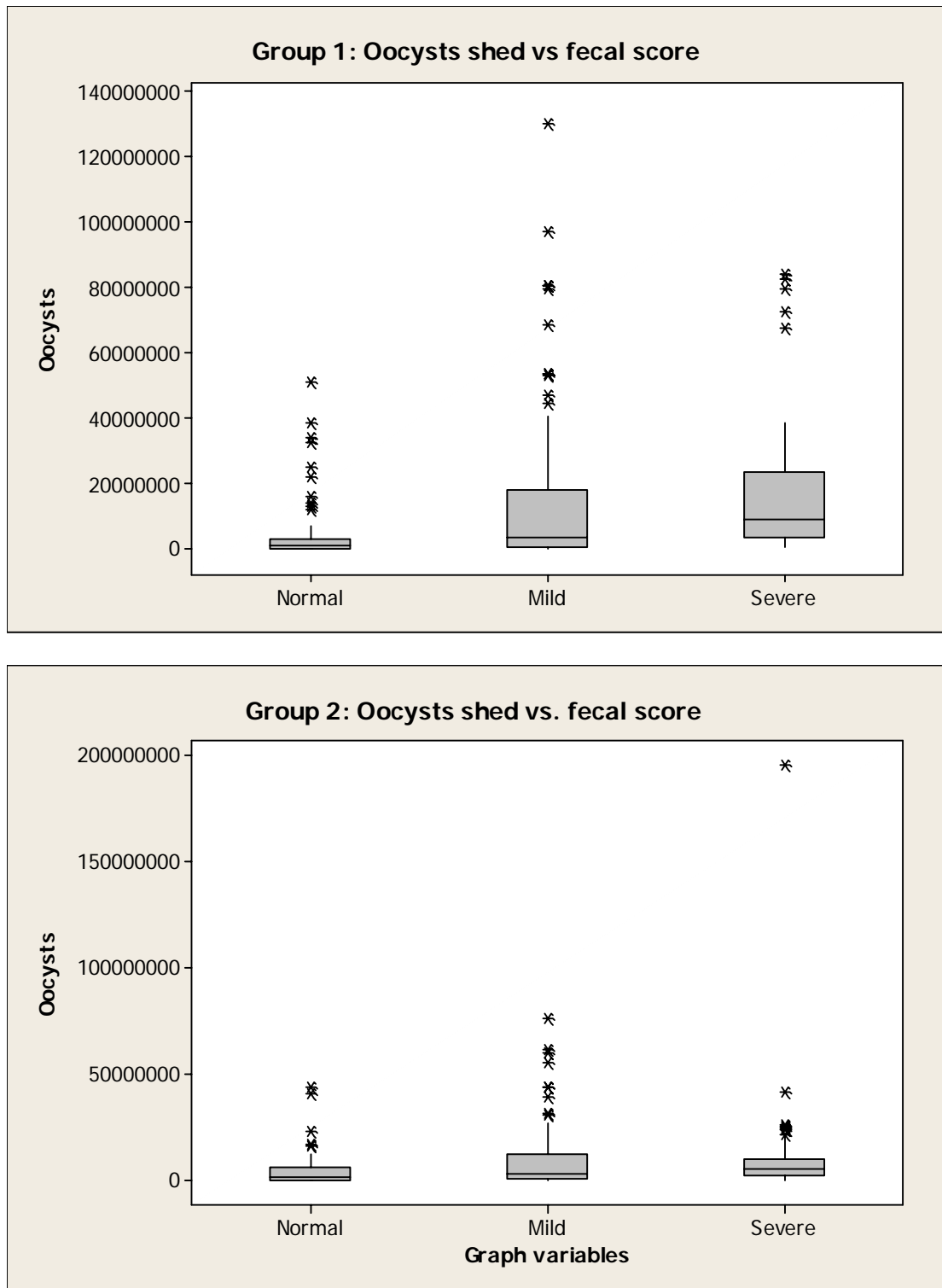


FIGURE 3. Box plots of oocysts shed for each fecal score for groups 1 and 2. Oocyst numbers for each fecal score category were plotted to show the minimum, maximum, median, quartiles 1 and 3, and outliers. (Normal = (1, 1.5), Mild diarrhea= (2, 2.5, 3), and Severe diarrhea = (3.5, 4)).

TABLE 1. Descriptive statistics of oocysts values for corresponding fecal scores in groups 1 and 2.

Group 1: Fecal Score	Sample Size (n)	Mean	Standard Deviation	Standard Error Mean	95% Confidence Interval
1	60	5.53×10^6	10.1×10^6	1.38×10^6	$(2.78 \times 10^6 - 8.28 \times 10^6)$
2	95	15.0×10^6	24.0×10^6	2.47×10^6	$(10.1 \times 10^6 - 19.9 \times 10^6)$
3	54	18.0×10^6	22.0×10^6	3.00×10^6	$(12.4 \times 10^6 - 24.4 \times 10^6)$

Group 2: Fecal Score	Sample Size (n)	Mean	Standard Deviation	Standard Error Mean	95% Confidence Interval
1	54	4.90×10^6	8.83×10^6	1.20×10^6	$(2.49 \times 10^6 - 7.31 \times 10^6)$
2	77	10.1×10^6	16.1×10^6	1.84×10^6	$(6.85 \times 10^6 - 14.2 \times 10^6)$
3	44	12.0×10^6	29.6×10^6	4.47×10^6	$(3.43 \times 10^6 - 21.4 \times 10^6)$

The number of oocysts shed by each animal, disregarding the feeding number, were categorized by their corresponding fecal score and grouped together. Values were obtained by running one sample t-tests on each category and displayed above.

TABLE 2. Association between oocyst shedding and fecal scores for groups 1 and 2.

Fecal Scores	Group 1, p-value	Group 2, p-value
FS 1 VS. FS 2	0.005	0.022
FS 1 VS. FS 3	0.000	0.079
FS 2 VS. FS 3	0.399	0.644

Using the oocysts values of each fecal score from table 1, the analysis of the means for each fecal score were compared amongst one another using ANOVA. The results obtained are listed above.

Conclusion:

After observing two separate, dissimilar groups of animals, this study revealed that an association exists between fecal score and oocyst shedding. While the two groups could not be compared against one another, within each group there were statistically elevated oocyst numbers in calves with diarrhea vs. normal fecal score. However, even though both groups of calves studied had increased numbers of oocyst shed with increasing fecal score, there was no statistical difference in the number of oocysts shed in calves with mild versus severe diarrhea. Although a rise in fecal score may be some indication that a calf is possibly shedding higher numbers of oocysts of *C. parvum*, visual observation of fecal matter alone may not be the best indication of the severity of infection. Previous studies have mentioned that severity of oocyst shedding is highly variable among calves (Olson et. al., 2004). Therefore, calf caregivers should always use precaution and practice good hygiene when working with calves so that infective oocysts are not spread to other animals, other workers or ground water supplies (Ollivett et. al., 2009).

Literature Cited:

- Castro-Hermida, J.A., Y. A. González-Losada, M. Mezo-Menéndez, and E. Ares-Mazás. A study of cryptosporidiosis in a cohort of neonatal calves. 2002. Vet. Parasitol. 106:11-17.
- Centers for Disease Control and Prevention. Cryptosporidiosis: life cycle. 2008. <http://www.cdc.gov/crypto/>.
- de Graff, D.C., E. Vanopdenbosch, L.M. Ortega-Mora, H. Abbassi, and J.E. Peeters. A review of the importance of cryptosporidiosis in farm animals. 1999. Int. J. Parasitol. 29:1269-1287.

- Despommier, D.D., R.W. Gwadz, P.J. Hotez, C.A. Knirsch. Parasitic diseases. 2006. 5:69-72.
- Fayer, R., L. Gasbarre, P. Pasquali, A. Canals, S. Almeria, and D. Zarlenga. *Cryptosporidium parvum* infection in bovine neonates: dynamic clinical, parasitic and immunologic patterns. 1998. Int. J. Parasitol. 28:49-56.
- Garber, L.P., M.D. Salman, H.S. Hurd, T. Keefe, and J.L. Schlater. Potential risk factors for *Cryptosporidium* infection in dairy calves. 1994. J. Am. Vet. Med. Assoc. 205:86-91.
- Harp, J.A., and J.P. Goff. Protection of calves with a vaccine against *Cryptosporidium parvum*. 1995. J. Parasitol. 81:54-57.
- Juranek, D.D. Cryptosporidiosis: sources of infection and guidelines for prevention. 1995. Clin. Infect. Dis. 21:S57-S61.
- MacKenzie, W.R., N.J. Hoxie, M.E. Proctor, M.G. Gradus, K.A. Blair, D.E. Peterson, J.J. Kazmierczak, D.G. Addiss, K.R. Fox, J.B. Rose, and J.P. Davis. A massive outbreak in Milwaukee of cryptosporidium infection transmitted through the public water supply. 1994. N. Engl. J. Med. 21(331):161-167.
- Nutrient Requirements of Dairy Cattle: 7th ed. 2001 by Committee on Animal Nutrition, National Research Council.
- Nydam, D.V. Calves with cryptosporidiosis can be frustrating: is there a treatment? 2003. Cceptor-Animal Health News. 11;13.
- Nydam, D.V., and H.O. Mohammed. Quantitative risk assessment of *Cryptosporidium* species infection in dairy calves. 2005. J. Dairy Sci. 88:3932-3943.
- Nydam, D.V., S.E. Wade, S.L. Schaaf, and H.O. Mohammed. Number of *Cryptosporidium parvum* oocysts or *Giardia spp* cysts shed by dairy calves after natural infection. 2001. AJVR. 62:1612-1615.
- Nydam, Personal Communication, unpublished data 2009.
- Ollivett, T. L., D.V. Nydam, D. D. Bowman, J. A. Zambriski, M.L. Belloso, T.C. Linden, and T.J. Divers. Effect of nitazoxanide on cryptosporidiosis in experimentally infected neonatal dairy calves. 2009. J. Dairy Sci. 92(4):1643-8.
- Olson, M.E., R.M. O’Handley, B.J. Ralston, T.A. McAllister, and R.C.A. Thompson. Update on *Cryptosporidium* and *Giardia* infections in cattle. 2004. Trends in Parasitol. 20:185-191.

- Saini, P.K., G. Ransom, and A.M. McNamara. Emerging public health concerns regarding cryptosporidiosis. 2000. JAVMA. 217:658-663.
- Singh, B.B., R. Sharma, H. Kumar, H.S. Banga, R.S. Aulakh, J.P.S. Gill, and J.K. Sharma. Prevalence of *Cryptosporidium parvum* infection in Punjab (India) and its association with diarrhea in neonatal calves. 2006. Vet. Parasitol. 140;162-165.
- Trotz-Williams, L.A., S.W. Martin, K.E. Leslie, T. Duffield, D.V. Nydam, and A.S. Peregrine. Association between management practices and within-herd prevalence of *Cryptosporidium parvum* shedding on dairy farms in southern Ontario. 2008. Prev. Vet. Med. 83;11-23.
- Tzipori, S., Cryptosporidiosis in animals and humans. 1983. Microbiol Rev. 47:84-96.
- Quiroz, E.S., C. Bern, J.R. MacArthur, L. Xiao, M. Fletcher, M.J. Arrowood, D.K. Shay, M.E. Levy, R.I. Glass, A. Lal. An outbreak of cryptosporidiosis linked to a foodhandler. 2000. J. Infect. Dis. 181:695-700.